TECHNICAL MANUSCRIPT 107

AUTOMATIC AMINO ACID ANALYSIS OF REPLICATE SAMPLES

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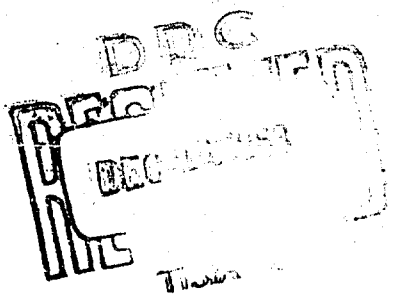

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ABSTRACT

A method is described by means of which replicate analyses of amino acids can be performed simultaneously, using an automatic amino acid analyzer. The limits to the number of samples that may be analyzed, and the minimal conditions for the application and separation of a given number of samples in a two-component mixture are discussed. This method has been applied to the determination of the C-terminal end-groups of proteins.

I. INTRODUCTION

Automatic amino acid analysis has been widely used for the quantitation of mixtures of amino acids. Customarily one sample is analyzed at a time, and the analysis may take many hours to complete. This report describes a method of analyzing replicate amino acid samples automatically and simultaneously.

II. METHODS

A sample containing 0.05 to 1.0 micromole of each component in pH 4.25 citrate buffer[1] was added to a resin column of a Phoenix Automatic Amino Acid Analyzer. After introduction of the sample and two 0.5 milliliter portions of buffer into the resin bed by air pressure, additional buffer was pumped through the column for 20 minutes under the usual operating conditions of column pressure, temperature, and buffer flow. The pump was then stopped, the column was opened, and another sample was applied in the same manner. Additional samples can be applied after subsequent 15- to 20-minute pumping periods. After the last sample had been run into the column, the ninhydrin pump was started, the ninhydrin was introduced into the effluent stream, and the recorder turned on.

III. RESULTS AND DISCUSSION

Samples containing only a single amino acid emerged as discrete, integratable peaks. By means of this procedure, as many as six replicate analyses of valine have been performed with a recovery of 100 ± 2 per cent. The only limits to the number of samples that may be applied to the column are (a) the complexity of the sample, (b) the length of the column, and (c) the rate of movement of the components on the column. Thus, three samples of a mixture of aspartic acid, alanine, and valine separated cleanly (pH 3.25 buffer, 150-centimeter column) but two samples of glycine and alanine overlapped because the rates of movement of these two substances through the column were too close.

The minimal conditions for the application and separation of n samples of a mixture containing components a and b on a column of length h may be expressed mathematically. Let the rates of movement of a and b in centimeters per minute be v_a and v_b where $v_b > v_a$. Let the distance from peak to peak of the components be p. From a practical standpoint the addition of n samples should be discontinued at time t when component b_1 is at h, so that the ninhydrin pump and recorder may be actuated to give a continous trace upon the chromatographic chart. In addition, the working value of p should be taken for that component emerging with the broadest band because p is a function of the time interval between applications and of the size of the sample. Thus the distance moved by b_1 during t is equal to $v_b t=h$. Component a_1 will be a distance Δh behind b_1. At the time t, it will have moved a distance $v_a t=h-\Delta h$. By equating t from both equations, it follows that $v_a/v_b=(h-\Delta h)/h$. The number of samples of b that can fit into the distance Δh is $n=\Delta h/p$. Substitution of this equation into the previous one gives the relationship $n=h(v_b-v_a)/v_b p$.

This method has been used to perform replicate analyses on protein samples degraded with hydrazine by the method of Akabori *et al*[3] to determine the C-terminal residue. The resulting free C-terminal amino acid was separated from accompanying hydrazides by reaction of the latter with benzaldehyde. The aqueous supernatant contained the C-terminal acid, ammonia, and traces of basic hydrazides emerging after the lysine peak upon amino acid analysis. The latter interfered with replicate determinations when the C-terminal residue was basic, but no difficulties were encountered when this amino acid appeared on the 150 centimeter column.

IV. SUMMARY

A simple method for replicate determinations of amino acids by automatic analysis is presented. It has been applied to the determination of the C-terminal end-groups of proteins.

LITERATURE CITED

1. Spackman, D.H.; Stein, W.H.; and Moore, S. "Automatic recording apparatus for use in the chromatography of amino acids," Anal. Chem. 30:1190-1205, 1958.

2. Akabori, S; Ohiro, K.; and Narita, K. "Hydrozinolysis of proteins and peptides: Method for the characterization of carboxyl terminal amino acids in proteins," Bull. Chem. Soc. Japan 25:214-218, 1952.